Impact of percutaneous pulmonary valve implantation procedural steps on leaflets histology and mechanical behaviour: An in vitro study

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ABSTRACT

Background: Percutaneous pulmonary valve implantation (PPVI) using bovine jugular vein Melody valve is safe and effective. However, post-procedural complications have been reported for unclear reasons.

Objective: We sought to assess the impact of PPVI procedural steps on valvular histology and leaflet mechanical behaviour.

Methods: Three different valved stents (Melody valve, homemade stents with bovine and porcine pericardium) were tested *in-vitro* in 4 conditions: I) control group, II) crimping, III) crimping + inflation of low-pressure balloon and IV) condition III + post dilatation (high-pressure balloon). For each condition, valvular leaflets (and venous wall sample for Melody stents) were taken for histological analysis and mechanical uniaxial tests of valve leaflets.

Results: Among Melody valves, incidence of transverse fractures was significantly higher in traumatized samples compared with control group ($p<0.05$) whereas, incidence and depth of transverse fractures were not statistically different between the 4 conditions for bovine and porcine pericardial leaflets. No significant modification in mechanical behaviour of *in-vitro* traumatized Melody® valvular leaflets was observed. Bovine and porcine pericardia became more elastic and less resilient after balloon expansion and post-dilatation (condition III and IV), with a significant decrease of elastic modulus and stress at rupture.

Conclusion: Valved stent implantation procedural steps induce histological lesions on Melody valve leaflets. Conversely, bovine and porcine pericardial valved stents were not histologically altered by in vitro manipulations although their mechanical properties were significantly modified. These data could explain some of the long-term complications observed with these substitutes.

Keywords: percutaneous valve, pulmonary valve, endocarditis.

RESUME

Introduction: Le remplacement valvulaire pulmonaire percutané utilisant le stent valvé Melody est efficace et sécurisé, cependant des endocardites infectieuses surviennent sur ces prothèses sans explication évidente.

But: Evaluer l'impact des manipulations pré-implantatoires sur la structure histologique et le comportement mécanique des feuillets valvulaires.

Méthodes : Nous avons testés *in-vitro* 3 types de stents valvés (prothèse Melody, stents valvés en péricarde bovin et porcin fabriqués manuellement) dans 4 conditions différentes : I) groupe témoin ne subissant aucune manipulation, II) sertissage sur un ballonnet III) sertissage + inflation du ballonnet à basse pression, IV) = groupe III + surdilatation par ballonnet à haute pression. A l'issu de chaque manipulation les feuillets valvulaires étaient prélevés sur les stents puis analysés sur le plan histologique et mécaniques (test de traction uniaxiale).

Résultats : Pour les valves Melody, on retrouvait plus de lésions histologiques sur les feuillets valvulaires dans les groupes II, III et IV par rapport au groupe contrôle ($p<0.05$). L'incidence de ces lésions n'était pas différente entre les 4 conditions pour les stents valvés péricardiques. Les propriétés mécaniques des valves Melody traumatisées n'étaient pas modifiées. Les péricardes bovin et porcin devenaient plus élastiques et moins résistants dans les conditions III et IV, avec une diminution du module d'élasticité et du stress à la rupture.

Conclusion : Les manipulations réalisées en salle de cathétérisme entraînent des lésions histologiques significatives sur les feuillets valvulaires des prothèses Melody. Les stents valvés en péricarde bovin et porcin ne sont pas altérés histologiquement par ces manipulations mais voient leurs propriétés mécaniques se modifier significativement. Ces données pourraient expliquées certaines complications observées à long terme avec ces substituts.

Mots clés: valve percutanée, valve pulmonaire, endocardite

List of abbreviations

RVOT: right ventricular outflow tract

BJV: Bovine jugular vein

PPVI: percutaneous pulmonary valve implantation

IE: infective endocarditis

BACKGROUND

Patients undergoing surgical right ventricular outflow tract (RVOT) reconstruction are subject to conduit degeneration later in life, requiring further interventions to alleviate the pulmonary stenosis and/or regurgitation that ensue. Since the first reported case in 2000, percutaneous pulmonary valve replacement (PPVI) using the Melody® valve (Medtronic Inc, Minneapolis, MN, USA) - a glutaraldehyde fixed bovine jugular vein (BJV) valve mounted on a balloon-expandable stent – is now recognized as an alternative to surgical pulmonary valve replacement in patients with failing RVOT [1,2]. Recent reports showed that PPVI was feasible at a relatively low risk and mid-term follow-up demonstrated a sustained improvement of haemodynamics up to 7 years after implantation [3,4]. Despite these promising results, various midterm and long-term complications have been described including cases of infective endocarditis (IE) [5-9]. The reported annualized rate of IE ranges from 2.4% to 3.9% per patient-year [10,11]. We and others recently showed that IE was more frequent after PPVI than surgical pulmonary valve replacement [11,12]. Infective endocarditis also involves other valved stents made with different valvular substrates – i.e. Edwards Sapien® (Edwards Lifesciences, Irvine, CA) made with bovine pericardium and Corevalve® (Medtronic Inc., Minneapolis, Minnesota, USA) made with porcine pericardium [13,14]. These results suggest that IE might be related to the implantation technique (i.e percutaneous or surgical) that is used for valvular placement. One of the main differences between surgical and transcatheter valve replacement is that percutaneous valves undergo several manipulations before (i.e. crimping) and during implantation (i.e. balloon expansion) whereas surgical prostheses are directly placed in the pulmonary pathway without theoretical valvular damage. Traumatic injury to biological valves leaflets has been reported during valved stents preparation [15,16]. In a recent work, we demonstrated that selective adhesion of S. aureus

and S. sanguinis pathogenic strains was noted on healthy Melody valve tissue and increased after implantation procedural steps [17].

In this *in-vitro* study we aimed to assess effects of PPVI procedural steps on histological and mechanical properties of Melody® valve leaflets and to compare these results with other tissues used for valved stents fabrication (i.e bovine and porcine pericardium).

METHODS

Valvular substrates

3 types of valved stents were tested experimentally:

1) The Melody® valve was obtained from Medtronic and stored in its commercial packaging.

2) Bovine pericardium: valvular leaflets were obtained from a bovine pericardial patch (10 x 15 cm - Edwards Lifescience, Irvine, USA), cut onto a 21-mm homemade 3 leaflets valvular mould and sutured into vascular stent (CP8Z34, Numed Inc, Canada). Valved stents were then stored in 0.625% glutaraldehyde until use.

3) Porcine pericardium: valvular leaflets were obtained from a porcine pericardial patch (8 x 6 cm - Vascutek Terumo Ltd., Swillington, Leeds). Porcine pericardial valved stents were then prepared similarly and stored in 0.625% glutaraldehyde until use.

In-vitro manipulations

For each valved stent, we compared 4 experimental conditions reproducing the sequential procedural steps leading to a conventional PPVI *(figure 1)*. Before manipulation, valved stents were rinsed twice for 2 minutes each in 500-ml saline baths to remove glutaraldehyde.

Condition I: Control group; valved stents were not manipulated.

Condition II: Compression group; valved stents were manually crimped on sterile syringes (5 and 2.5-ml) and then onto the 22-mm balloon of the 22-French Ensemble® delivery system (*figure 1A)*. The sheath was advanced to cover the balloon-mounted valved stent during 5 minutes. This duration was chosen arbitrarily and aims to reproduce the crimping duration during a conventional PPVI. The compressed prostheses were regularly flushed with a saline solution. The sheath was then drawn back and the valved stent was manually enlarged and removed avoiding damage on the valve.

Condition III: Compression/Expansion group; valved stents were first prepared as in condition II. After the sheath was drawn back, valved stents were deployed in a 20-mm Goretex conduit by inflation of the inner and outer balloons of the delivery system. The balloons were then deflated and the delivery system removed (*figure 1A to B*).

Condition IV: Compression/expansion/post-dilatation; valved stents were first prepared as in condition III. After valve deployment, a post-dilatation using a 22-mm high-pressure balloon (Atlas Gold, Bard Peripheral Vascular, Inc., Tempe, AZ, USA) inflated at 20 ATM for 5 seconds was performed (*figure 1A to C*).

Valved stents were analysed in each *in-vitro* condition for a same substrate. For each Melody® valved stent in each condition, 3 additional samples of the BJV wall adjacent to the leaflets within sinuses were taken using an 8-mm diameter (i.e. 0.5 cm^2) trepan for histological tests. After sampling, valvular leaflets and BJV wall fragments were stored in 0.625% glutaraldehyde until histological processing (within 24 hours) or in a saline solution before an immediate mechanical testing *(figure 1D).*

For each substrate five leaflets coming from 2 valved stents were studied for histological and mechanical evaluation in each of the 4 conditions.

Uniaxial tensile test

We determined leaflets mechanical properties using uni-axial tensile tests with a universal testing machine Adamel Lhomargy MTS 100 (MTS Systems Corporation; Eden Prairie, MN) equipped with test TestWorks 4 software (MTS Systems Corporation; Eden Prairie, MN). The mechanical properties of native or prosthetic valvular leaflets have been published previously with validated methods [18,19].

Five leaflets were tested for each substrate in each of the 4 conditions. Tissue thickness was

measured with a caliper with a precision of 0.01 mm. A 100 N load cell was used to apply a tensile force to the tissue samples, and the tissue was stretched at a constant rate of 0.5 mm/min to obtain stress-strain curve on which were recorded stress at break, elongation at break, ultimate tensile strength (maximum stress that a material can withstand while being stretched before breaking) and elastic modulus (slope of stress–strain curve in the elastic deformation region).

Histological analysis

Macroscopic analysis preceded microscopic evaluation. After paraffin embedding, 5-µm thick samples were stained with haematoxylin and eosin (H&E) and digitalized pictures obtained at x5 and x20 magnifications. Transverse tissue fracture, the basic lesion previously described for bovine pericardial valved stents originated from one surface of the sample deep inside the tissue [15]. It was considered as arbitrarily significant when its depth exceeded 25% of sample's thickness. The depth of the biggest fracture was calculated as a percentage (fracture's length/sample thickness). The number of fractures and the depth of the biggest fracture were determined at x5 magnification. The pathologist was blinded for the type of *in-vitro* manipulation that the sample underwent.

Statistical analysis

Results were expressed as mean (standard deviation) or median (range) for continuous variables or as a number (percent) for categorical variables. The data from these experiments were analysed with nonparametric Mann–Whitney or Kruskal-Wallis tests. These tests were performed to compare variables between 2 groups (i.e. condition I vs II ; I vs III ; I vs IV ; II vs III ; etc.). The value of statistical significance was set at $p \leq 0.05$.

RESULTS

Uniaxial tensile tests

Bovine, porcine and Melody® valve leaflets had a median thickness respectively of 0.57-mm (0.45 – 0.7), 0.23-mm (0.18 – 0.25) and 0.1-mm (0.08 – 0.1) and a median width respectively of 8-mm (5 – 12), 9-mm (5 – 11) and 6-mm (5-9). Uniaxial measurements showed typical non-linear J-shaped stress-strain curves. *Table 1* shows the mechanical behaviour of each substrate obtained in the different *in-vitro* conditions.

No statistical difference was observed for the *Melody® valvular leaflets* between the 4 different conditions, for each studied parameter (stress and elongation at break, ultimate tensile strength, and elastic modulus).

Concerning *bovine pericardial valved stents*, stress at break was significantly lower in conditions III and IV compared with control condition (I vs III: p=0.02; I vs IV: p=0.005). Elastic modulus was significantly lower in condition III compared to condition I and II (I vs III: p=0.042; II vs III: p=0.001). No difference was observed between conditions III and IV for elastic modulus values.

Concerning *porcine pericardial valved stents*, stress at break and ultimate tensile strength were significantly lower in condition IV compared to control condition I (p=0.032 and 0.03 respectively). Elastic modulus was significantly lower in condition III and IV compared to condition I (I vs III: p=0.04; II vs III: p=0.031).

Histological analysis

No macroscopic lesion such as perforation, tear or laceration of the leaflets (or venous wall for Melody® valved stent) was observed, whatever the *in-vitro* condition was. Microscopic analysis revealed presence of transverse fractures in all valvular leaflets (Melody® valve, bovine pericardium, porcine pericardium) but not in Melody® BJV wall samples *(figure 2*

and 3). Histological lesions had a heterogeneous distribution. Indeed, areas of healthy tissue surrounded areas of severely traumatized tissue within a same leaflet. Except for porcine pericardium, transverse fractures were rarely found in samples from control groups (condition I). Histological lesions according to substrate and *in-vitro* conditions are presented in *table 2*.

Among *Melody® valvular leaflets*, the incidence of transverse fractures was significantly higher in traumatized samples compared with control group (I vs II: p=0.043; I vs III: p=0.043 and I vs IV: p=0.042). No difference was observed between conditions II, III and IV. Transverse fractures were significantly deeper in compression group (I vs II: p=0.042).

Among *bovine and porcine pericardial leaflets*, the incidence and depth of transverse fractures were not statistically different between the 4 *in-vitro* conditions.

No histological lesion was observed in BJV wall samples whatever the condition was.

DISCUSSION

The aims of this study were 1) to assess the impact of implantation procedural steps on histological structure and mechanical behaviour of the Melody transcatheter pulmonary valved stent leaflets and 2) to compare these results with other valvular substrates such as bovine and porcine pericardia.

Among Melody prostheses, we found that procedural steps resulted in significant histological lesions from the crimping and compression stage. Conversely, bovine and porcine pericardial valved stents were not histologically altered by in vitro manipulations although their mechanical properties were significantly modified.

Histological lesions are induced from the early steps of valved stent implantation

Amahzoune et al. showed that histological lesions such as transverse fractures and longitudinal cleavages occurred during crimping and deployment, with more severe injuries induced by balloon-expandable valved stents [15]. These results suggested a cumulative impact of both crimping and balloon expansion stages on valvular leaflets architecture. Other authors observed comparable lesions with bovine pericardial valved stent using various techniques of assessment [16-20]. Unlike Amahzoune et al. results, we did not found that bovine pericardial leaflets were significantly injured during *in vitro* manipulations [15]. This difference can be explained by the fact that fresh bovine pericardium obtained from a slaughterhouse was used in their study while we performed our tests with commercially treated and thus possibly more resistant bovine pericardium. Furthermore, the stents were not the same in the 2 studies: the platinum and iridium CP8Z34 (NuMED, Inc., Hopkinton, New York, USA) stent is more flexible and less sharp than the stainless steel stent used by Amahzoune et al. Finally, the fact that we analysed less samples than these authors can partially explain the absence of statistically significant results for this substrate.

We observed transverse fractures lesions in all traumatized substrates except Melody jugular venous wall. In our analysis we did not include the presence of longitudinal cleavages, which are possibly non-specific and may correspond to artefacts. We showed that incidence and depth of transverse fractures were significantly higher in traumatized samples compared with control Melody valvular leaflets group. No difference was observed between conditions II, III and IV. Moreover, it is noteworthy that traumatized Melody jugular venous wall was free of histological lesions These results suggest that lesions appear from the early implantation procedural step (crimping/compression) without cumulative impact of balloon expansion or postdilatation. Crushing and shearing of valvular leaflets between the stent on one side and the balloon on the other hand during crimping and balloon inflation may explain these lesions. Schneider et al. performed an *ex-vivo* assessment of 9 percutaneously implanted valved Melody conduits after surgical explantation by means of histology and immunohistochemistry [21]. The authors found that, in the absence of infection, the valve cusps were clinically competent and histologically thin and intact. This is not consistent with our findings however, the authors did not focus on the presence of transverse fractures. In addition, although valvular fractures are induced in the early post-implantation period, *in-vivo* neo-endothelialization might be beneficial for these lesions by homogenizing the leaflets surface.

One could argue that leaflets traumatic injury observed our experiment might theoretically lead to an accelerated deterioration of valvular prostheses. Indeed, fractures of collagen bundles may create new sites for calcium deposition and thus decrease life duration of the valve [22]. However, after a mean implantation time of 3.2 years, Schneider et al. observed a complete neo-endothelialization for all specimens without significant pseudointimal proliferation, and without calcifications within the valves. To date, there are no published data reporting early calcification or degeneration of the Melody valve in the pulmonary position. Although early failure of Melody valves implanted within bioprosthetic tricuspid valves has

been reported, the pathophysiology of these isolated cases remained unclear [21,23]. Furthermore, the mid-term valvular function of the Melody valve is encouraging although long-term data are lacking [3,4].

Mechanical behaviour of leaflets after traumatic injury

Soft biological tissues have specific mechanical properties. Mechanical behaviour is one the key characteristics of cardiac valve function. These properties are mainly determined by valve tissular architecture and by the balance between extracellular matrix components [24].

In our experiment, we found no significant modification in mechanical behaviour of *in-vitro* traumatized Melody® valvular leaflets. However, we can also conclude that BJV leaflets are a resilient substrate slightly affected by traumatic injury. Munelly et al. analysed mechanical behaviour of bovine pericardium after compression under forces similar to those exerted by valved stent crimping. They showed that bovine pericardium was significantly stiffened by this process with an increase in elastic modulus [25]. We also found an increase in bovine pericardium elastic modulus in our compression group (condition II). Conversely, this substrate became more elastic and less resilient after balloon expansion and post-dilatation (condition III and IV), with a significant decrease of elastic modulus and stress at rupture.

Similar results were observed with porcine pericardial stents, the latter becoming more elastic and less resilient after balloon expansion and post-dilatation. It is noteworthy that there was no difference between conditions III and IV, showing that there was no cumulative impact of post-dilatation.

Clinical implications

Our findings should be analysed in light of published data on incidence and of right-sided endocarditis in patients with congenital heart disease after surgery or PPVI. The role played

by BJV in endocarditis pathophysiology is concerning. We clearly demonstrated for the first time that BJV leaflets were the most histologically altered by *in-vitro* manipulations. In a recent work, we hypothesized that the transverse fractures observed on traumatized leaflets might constitute a possible target for bacterial germs to adhere and possibly explain the high incidence of IE involving Melody valves [17, 26-28]. However, scanning electron microscopy revealed that, bacteria adhered over the entire sample surface in a heterogeneous way. Nevertheless, we suggested that histological traumatic lesions might increase surface roughness by modifying topographical characteristics of the sample, and thus enable higher microbial adhesion [17].

We showed that incidence of transverse fractures were significantly higher in traumatized samples compared with control Melody valvular leaflets group. No difference was observed between conditions II, III and IV. These results suggest that lesions appear from the early implantation procedural step (crimping/compression) without cumulative impact of balloon expansion or postdilatation. These results highlight the fact that traumatic leaflets lesions would not necessarily be avoided by the use of a valve mounted on a self-expanding stent, as crimping is also mandatory with such a device.

Moreover, the usefulness of post-dilation is debated in clinical practice for PPVI. In this experiment, we showed that, regardless to the type of valvular substrate, postdilatation was not deleterious whether in terms of histological lesions or in terms of mechanical behaviour.

It is still unclear whatever the incidence of endocarditis is higher with bovine jugular vein compared to pericardium valves in clinical practice. Results of clinical study from Edwards pulmonic valve are still missing. However, two points should be taken into account when comparing data. First, results from initial US cohort reported only orally by Pr Hijazi ZM in various meetings (SCAI 2014, PICS 2014, 2015) showed an incidence of endocarditis of 3.2% at one year (2 endocarditis over a cohort of 63 patients). Finally, population should be

similar when comparing data. Indeed, from unpublished clinical data, included population varied greatly between the 2 cohorts. With Melody, patients included tended to have smaller conduits with stenotic and pulmonary regurgitation when Edwards patients had mostly large unobstructed right ventricular outflow tract. This could artificially decrease the incidence of endocarditis as it has been reported that patients with pure pulmonary regurgitation and low right ventricular outflow tract gradient after Melody implantation have less endocarditis. Larger comparative studies are therefore necessary before drawing any conclusion.

Limitations

Our study suffers from limitations. The small number of studied sample in each subgroup may have reduced statistical significance of our results. Uniaxial tensile tests were only performed in the radial direction of the samples while they are known to have an anisotropic behaviour. In our experiment, we induced *in vitro* acute lesions and our model did not take into account the role of cardiac output, pressure gradients and shear stress forces.

CONCLUSION

Valved stents implantation procedural steps induce Melody® valve leaflets histological lesions right from the crimping stage. Conversely, bovine and porcine pericardial valves are not significantly altered by procedural manipulations on histological analysis. Mechanical properties of Melody® valve leaflets, the thinnest of all, are not altered by these manipulations whereas bovine and porcine pericardial valves are significantly modified. This could have an impact on long term valvular function and durability of pericardial valve. In the other hand, despite reassuring data on mechanical properties with Melody valves, the histological lesions observed on traumatized leaflets might constitute a possible target for bacterial germs and possibly explain the high incidence of IE involving Melody valves. Further experimental studies are warranted to better understand this issue. Results from

clinical studies with pericardial valves focusing on endocarditis issue are obviously needed to demonstrate any propensity of a tissue over another to infective endocarditis.

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FIGURE LEGENDS

Figure 1. **Description of *in vitro* manipulations realised on Melody® valved stent**

A: Melody® valved stent after saline solution rinsing; B and C: crimping on sterile 5-mL and 2.5-mL syringes; D: crimping on Ensemble® delivery system ; E: covering of the valved stent by the sheath; F: expansion of the valved stent in a 20-mm Goretex conduit ; G: post-dilatation using high pressure balloon ; H: bovine jugular vein detached from the stent and opened on its longitudinal axis, note the 3 valvular leaflets; I: one valvular leaflet after sampling.

Figure 2. Histological observation of Melody® valvular leaflets (H&E staining; x 5 magnification)

A: microscopic aspect of a control valvular leaflet (condition I) with a conserved architecture; B: microscopic aspect of an injured valvular leaflet (condition III - expansion), note the inhomogeneous architecture associated to a large transverse fracture (*).

Figure 3. Histological observation of bovine (A&B) and porcine (C&D) pericardial leaflet pericardial leaflets (H&E staining; x 5 magnification)

A: microscopic aspect of a control bovine pericardial valvular leaflet (condition I) with a preserved organization of collagen bundles; B: microscopic aspect of a traumatized bovine pericardial valvular leaflet (condition IV – post-dilatation), note the complete architecture alteration associated with a large transverse fracture (*); C: microscopic aspect of a control porcine pericardial valvular leaflet (condition I) with a slightly altered architecture; D: microscopic aspect of an injured porcine pericardial valvular leaflet (condition III - expansion), note the presence a large transverse fracture (*).

TABLES

Table 1. Mechanical properties of the different valvular samples

Melody ® Leaflet	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Postdilatation (n=5)**	***p* values**
Stress at break (MPa*)	5.7 (2.6-8)	5.7 (2.8-7.9)	4.6 (3.7-13)	5.5 (3.7-11.5)	I vs II, p=0.48 I vs III, p=0.89 I vs IV, p=0.87 II vs III, p=0.66 II vs IV, p=65 III vs IV, p=0.79
Elongation at break (%)	34.6 (31-54)	44 (32-49)	66 (19-83)	58 (30-67)	I vs II, p=0.74 I vs III, p=0.45 I vs IV, p=0.38 II vs III, p=0.48 II vs IV, p=43 III vs IV, p=0.7
Ultimate tensile strength (MPa)	5.7 (2.7-8.8)	5.6 (2.5-8.9)	5.2 (3.7-13)	5.6 (3.9-11.8)	I vs II, p=0.64 I vs III, p=0.68 I vs IV, p=0.66 II vs III, p=0.67 II vs IV, p=0.65 III vs IV, p=0.79
Elastic modulus (MPa)	0.16 (0.1-0.4)	0.19 (0.18-0.4)	0.2 (0.16-0.32)	0.24 (0.18-0.31)	I vs II, p=0.34 I vs III, p=0.91 I vs IV, p=0.71 II vs III, p=0.71 II vs IV, p=0.90 III vs IV, p=0.14

Bovine pericardium	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Postdilatation (n=5)**	***p* values**
Stress at break (MPa)	8.7 (8.2-10)	10.2 (7-12.9)	6 (5-8)	7 (5.7-9.1)	I vs II, p=0.5 *I vs III, p=0.02* *I vs IV, p=0.005* II vs III, p=0.18 II vs IV, p=0.5 III vs IV, p=0.30
Elongation at break (%)	45 (39-61)	48 (24-60)	45.8 (40-49)	42.6 (35-58)	I vs II, p=0.72 I vs III, p=0.62 I vs IV, p=0.58 II vs III, p=0.84 II vs IV, p=0.99 III vs IV, p=0.75
Ultimate tensile strength (MPa)	9.7 (6.3-12)	10.7 (7.2-14)	7 (6.3-8.6)	8.4 (6-10.2)	I vs II, p=0.85 I vs III, p=0.08 I vs IV, p=0.13 II vs III, p=0.14

Elastic modulus (MPa)	0.42 (0.37-0.54)	0.52 (0.41-0.54)	0.36 50.32-0.38)	0.38 (0.33-0.58)	II vs IV, p=0.12 III vs IV, p=0.37 I vs II, p=0.33 *I vs III, p=0.042* I vs IV, p=0.19 *II vs III, p=0.001* II vs IV, p=0.22 III vs IV, p=0.43

Porcine pericardium	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Postdilatation (n=5)**	***p* values**
Stress at break (MPa)	9 (7.5-10.8)	9.4 (7.1-12)	7.6 (2.8-8)	4.9 (1.7-7)	I vs II, p=0.35 I vs III, p=0.15 *I vs IV, p=0.032* II vs III, p=0.06 II vs IV, p=0.33 III vs IV, p=0.28
Elongation at break (%)	31 (25-36)	29 (21-37)	36 (32-58)	24 (20-35)	I vs II, p=0.54 I vs III, p=0.17 I vs IV, p=0.25 II vs III, p=0.17 II vs IV, p=0.66 III vs IV, p=0.09
Ultimate tensile strength (MPa)	9.2 (7.6-11)	10.5 (7-12)	8.2 (3-8.7)	5 (2-7)	I vs II, p=0.41 I vs III, p=0.24 *I vs IV, p=0.03* II vs III, p=0.12 II vs IV, p=0.27 III vs IV, p=0.23
Elastic modulus (MPa)	0.49 (0.4-0.8)	0.7 (0.36-1)	0.31 (0.24-0.44)	0.43 (0.15-0.46)	I vs II, p=0.28 *I vs III, p=0.04* *I vs IV, p=0.031* II vs III, p=0.35 II vs IV, p=0.31 III vs IV, p=0.92

Median (range), *: Mega-Pascal.

Table 2. Histological lesions according to substrate and *in-vitro* conditions.

Melody® Leaflet	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Post dilatation (n=5)**	***p* values**
Transverse fracture	3 (1-4)	8 (5-18)	11 (5-23)	7 (6-21)	*I vs II, p=0.043* *I vs III, p=0.043* *I vs IV, p=0.042* II vs III, p=0.71 II vs IV, p=0.17 III vs IV, p=0.78
Deepest fracture	0.4 (0.26-0.93)	0.92 (0.62-1)	0.68 (0.5-0.81)	0.87 (0.6-1)	*I vs II, p=0.042* I vs III, p=0.13

					I vs IV, p=0.08 II vs III, p=0.42 II vs IV, p=0.68 III vs IV, p=0.13

Bovine pericardium	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Post dilatation (n=5)**	***p* values**
Transverse fracture	0 (0-1)	0 (0-1)	1 (0-6)	0 (0-1)	I vs II, p=0.31 I vs III, p=0.18 I vs IV, p=0.31 II vs III, p=0.10 II vs IV, p=1 III vs IV, p=0.10
Deepest fracture	0 (0-0.38)	0 (0-0.26)	0.28 (0.26-0.48)	0 (0-0.42)	I vs II, p=0.14 I vs III, p=0.14 I vs IV, p=0.65 II vs III, p=0.06 II vs IV, p=0.17 III vs IV, p=0.14

Porcine pericardium	**Condition I Control (n=5)**	**Condition II Compression (n=5)**	**Condition III Expansion (n=5)**	**Condition IV Postdilatation (n=5)**	***p* values**
Transverse fracture	7 (0-9)	2 (0-6)	4 (1-16)	6 (2-8)	I vs II, p=0.41 I vs III, p=0.46 I vs IV, p=0.85 II vs III, p=0.41 II vs IV, p=0.08 III vs IV, p=1
Deepest fracture	0.27 (0-0.86)	0.4 (0-0.68)	0.48 (0.39-0.72)	0.42 (0.38-0.64)	I vs II, p=0.68 I vs III, p=0.34 I vs IV, p=0.5 II vs III, p=0.22 II vs IV, p=0.5 III vs IV, p=0.06

Median (range); deepest fracture: fracture's length/sample thickness

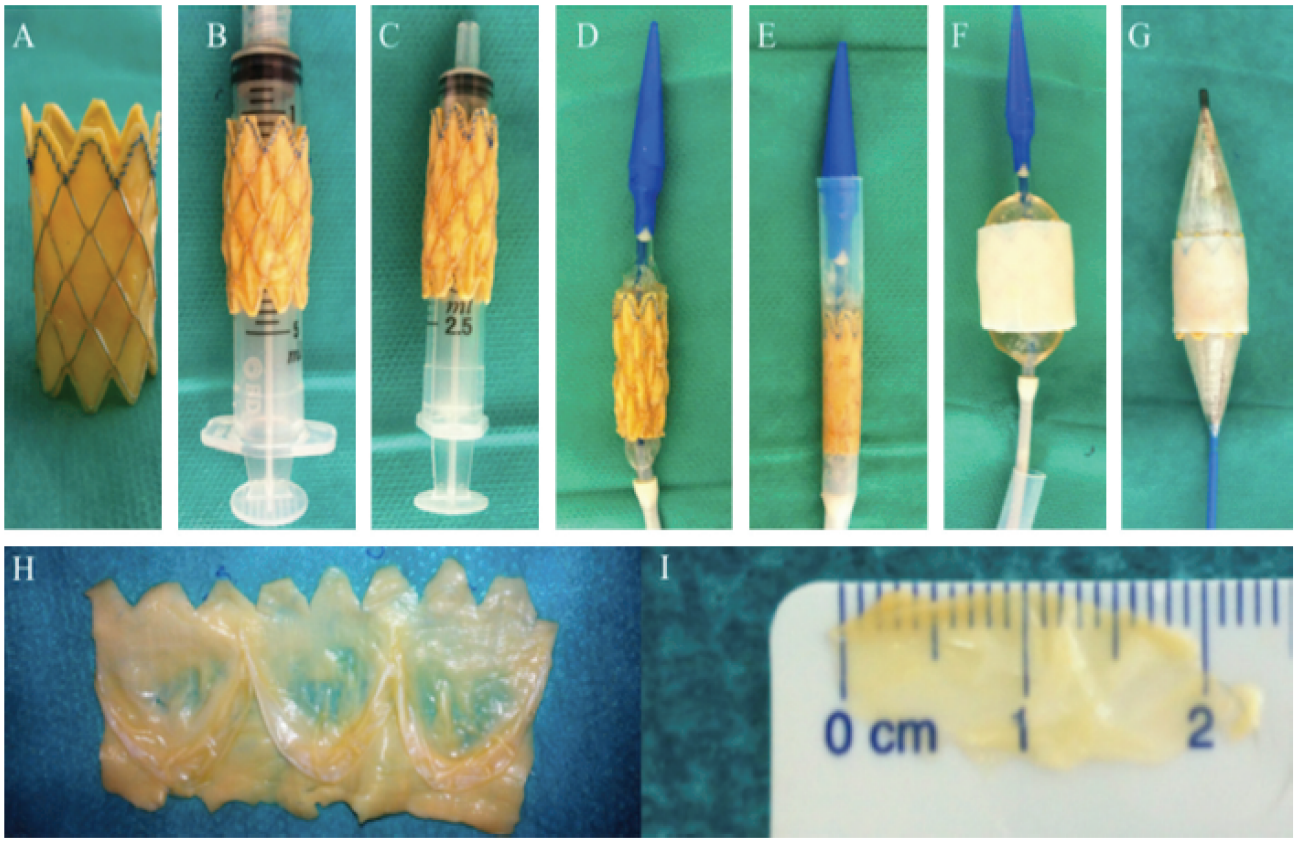

Figure 1

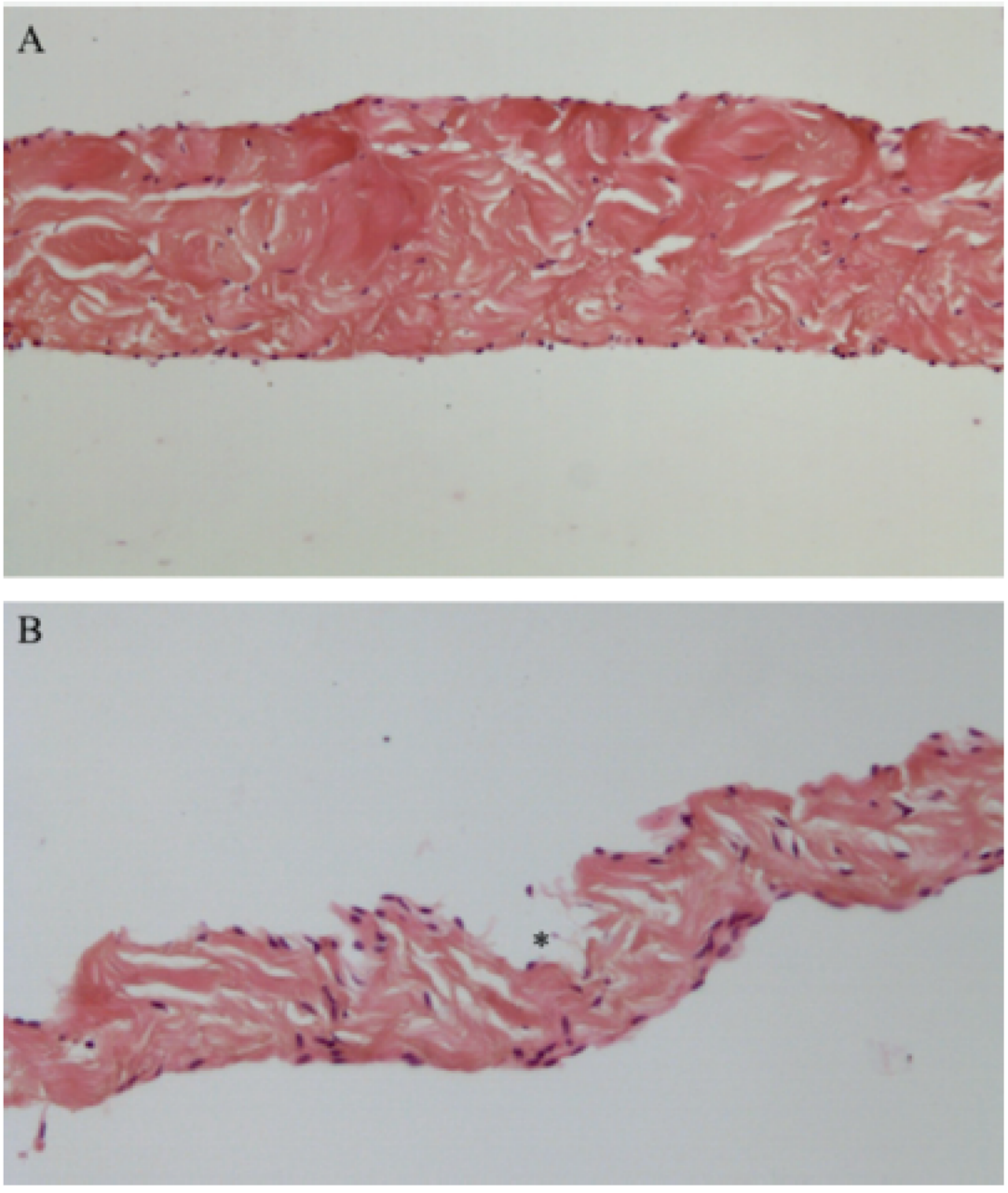

Figure 2

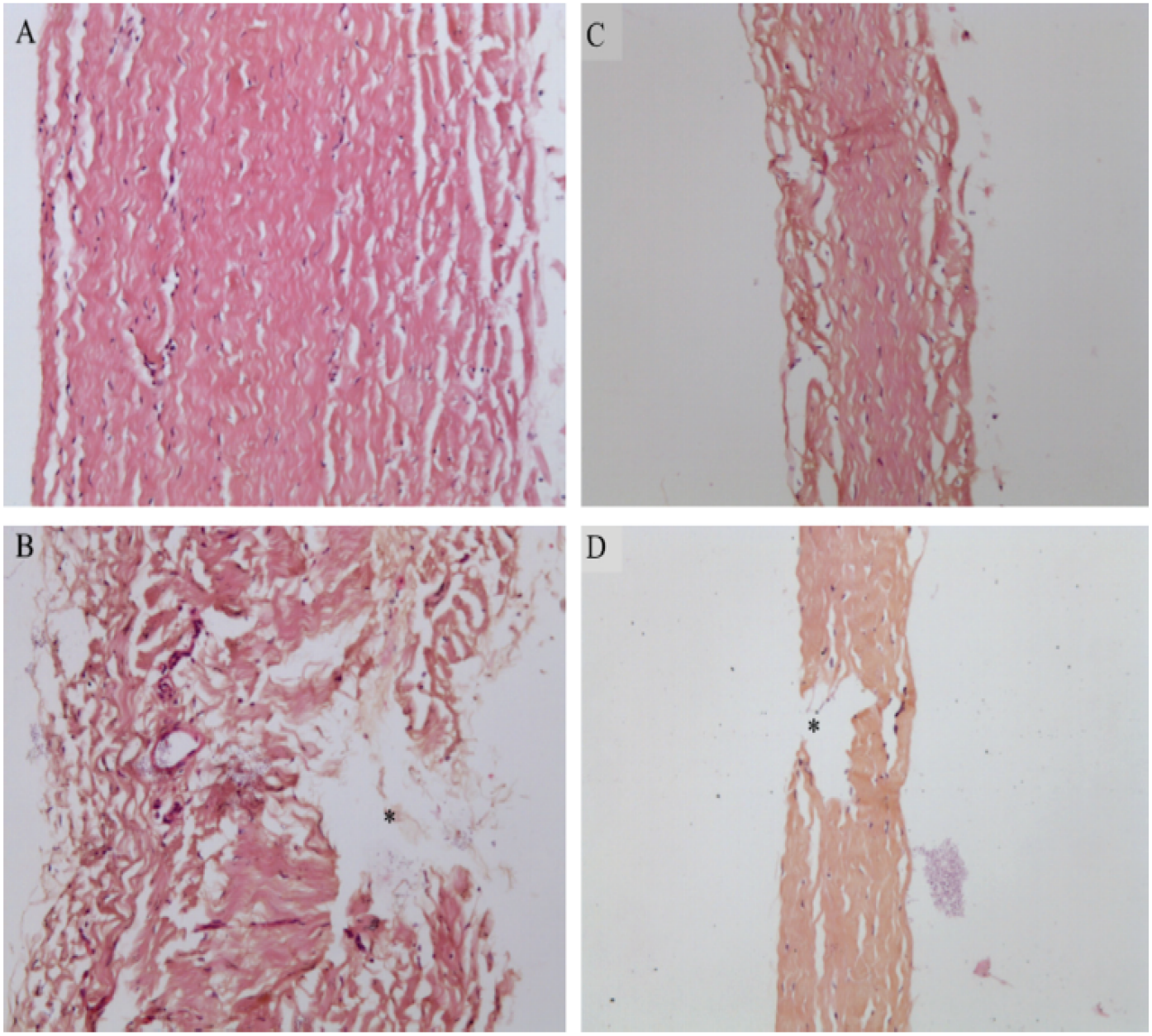

Figure 3